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Comparison of the DiversiLab Repetitive Element PCR System with *spa* Typing and Pulsed-Field Gel Electrophoresis for Clonal Characterization of Methicillin-Resistant *Staphylococcus aureus*[▽]

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The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has become an increasing problem worldwide in recent decades. Molecular typing methods have been developed to identify clonality of strains and monitor spread of MRSA. We compared a new commercially available DiversiLab (DL) repetitive element PCR system with *spa* typing, *spa* clonal cluster analysis, and pulsed-field gel electrophoresis (PFGE) in terms of discriminatory power and concordance. A collection of 106 well-defined MRSA strains from our hospital was analyzed, isolated between 1994 and 2006. In addition, we analyzed 6 USA300 strains collected in our institution. DL typing separated the 106 MRSA isolates in 10 distinct clusters and 8 singleton patterns. Clustering analysis into *spa* clonal complexes resulted in 3 clusters: *spa*-CC 067/548, *spa*-CC 008, and *spa*-CC 012. The discriminatory powers (Simpson's index of diversity) were 0.982, 0.950, 0.846, and 0.757 for PFGE, *spa* typing, DL typing, and *spa* clonal clustering, respectively. DL typing and *spa* clonal clustering showed the highest concordance, calculated by adjusted Rand's coefficients. The 6 USA300 isolates grouped homogeneously into distinct PFGE and DL clusters, and all belonged to *spa* type t008 and *spa*-CC 008. Among the three methods, DL proved to be rapid and easy to perform. DL typing qualifies for initial screening during outbreak investigation. However, compared to PFGE and *spa* typing, DL typing has limited discriminatory power and therefore should be complemented by more discriminative methods in isolates that share identical DL patterns.

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) in recent decades has become an increasing problem in healthcare settings and communities worldwide. MRSA infections have increased steadily since the first reported case in 1961, and MRSA infection is associated with worse outcomes and higher costs for care compared to methicillin-sensitive *S. aureus* infections (25).

Therefore, MRSA control is a cornerstone of any infection control program. Surveillance determines background rates and allows the rapid detection of clusters and outbreaks. However, typing methods have also become an indispensable tool for distinguishing clonal nosocomial clusters from unrelated increase of MRSA rates. Several genetic typing methods for the clonal characterization of isolates have been developed. Macrorestriction pattern analysis using pulsed-field gel electrophoresis (PFGE) was one of the first genome-based typing methods for MRSA (2), followed by repetitive element PCR typing (rep-PCR) (6), *spa* sequence typing (9), multilocus sequence typing (MLST) (7), and multilocus variable tandem repeat analysis (12). PFGE is often considered the gold standard for typing MRSA isolates in epidemiological studies. Although this method is known to be highly discriminatory, it is technically demanding and time-consuming, it has a low throughput, and its technical instability has adverse effects on reproducibility (21, 30, 34). The determination of sequence polymorphism in the variable X region of the *spa* gene encoding the *S. aureus* surface protein A (*spa* typing) has become one of the primary typing methods for regional and national MRSA surveillance programs. The advantages of this method are its excellent interlaboratory reproducibility, high throughput, and strong discriminatory power (1, 16).

The DiversiLab (DL) typing system (bioMérieux, Geneva, Switzerland) is a relatively new, commercially available rep-PCR typing method that uses the presence of repetitive sequences present in the organism's genome to determine the genetic relatedness of bacterial and fungal isolates (14). The method provides standardized and very rapid results. It benefits from the higher resolution of using a capillary electrophoresis methodology over standard gel-based techniques, uses modern web-based data analysis tools, and is easy to perform (10, 24, 26, 31, 32).

The aim of our study was to evaluate the performance and feasibility of the DL method compared to the well-known, established typing methods PFGE and *spa* typing. For the first time, we also analyzed and compared the clonal clustering of determined *spa* types by using the "based upon repeat pattern" (BURP) algorithm. We used a collection of 106 well-defined MRSA strains from our hospital (8). Quantitative data concerning discriminatory power and concordance of the four typing methods were calculated to validate and discuss the

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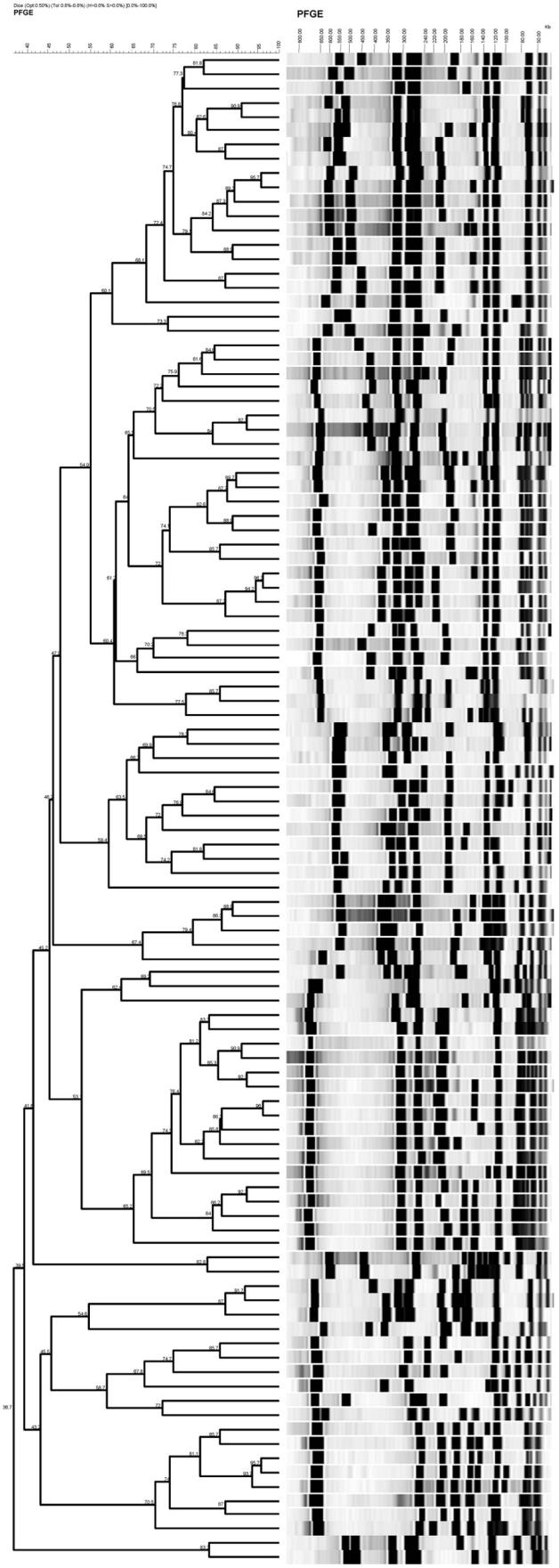
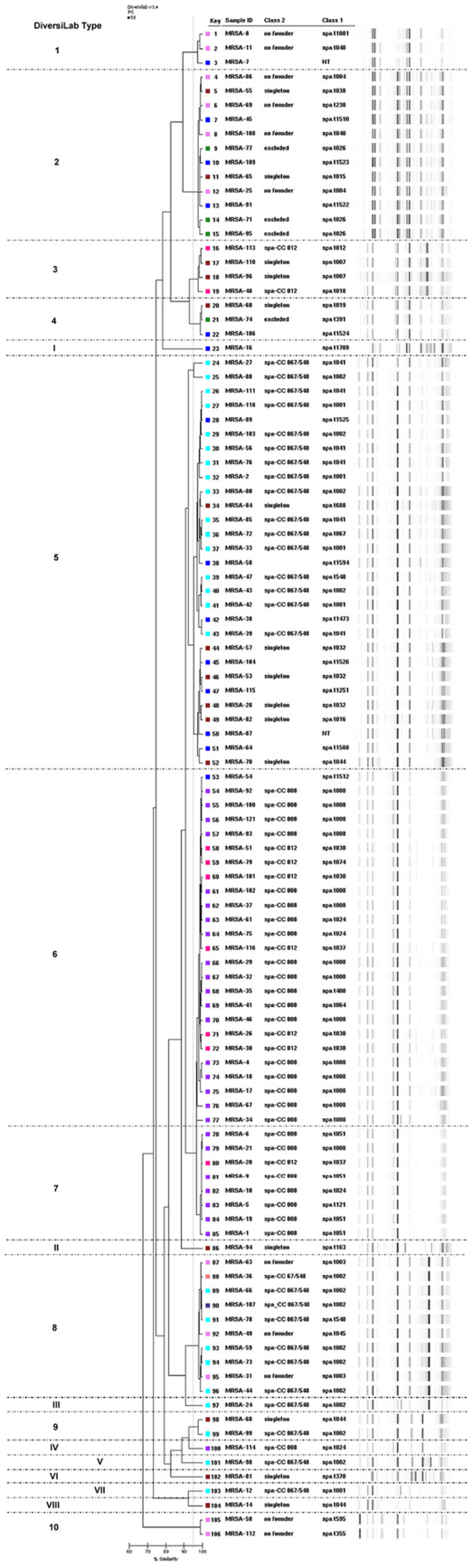


DiversiLab	PFGE	SPA	Kreiswirth	SPA-CC	Sample ID
5	1	t1473	TJMBMBMGMK	without alignment	MRSA-38
5	1	t1594	TJMBMD[r133]GMK	without alignment	MRSA-58
5	2	t548	TJMBMDMGK	067/548	MRSA-47
5	3	t002	TJMBMDMGMK	067/548	MRSA-88
5	3	t002	TJMBMDMGMK	067/548	MRSA-103
5	3	t002	TJMBMDMGMK	067/548	MRSA-80
8	3	t003	TMDMGMMK	no founder	MRSA-63
8	3	t548	TJMBMDMGK	067/548	MRSA-78
8	4	t002	TJMBMDMGMK	067/548	MRSA-36
8	4	t002	TJMBMDMGMK	067/548	MRSA-73
III	4	t002	TJMBMDMGMK	067/548	MRSA-24
8	4	t002	TJMBMDMGMK	067/548	MRSA-66
9	4	t002	TJMBMDMGMK	067/548	MRSA-99
8	5	t002	TJMBMDMGMK	067/548	MRSA-44
V	5	t002	TJMBMDMGMK	067/548	MRSA-98
8	6	t003	TMDMGMMK	no founder	MRSA-31
8	6	t002	TJMBMDMGMK	067/548	MRSA-59
5	7	t688	TJMBMK	singleton	MRSA-84
5	8	t067	TJMBMDMGM	067/548	MRSA-72
8	9	t002	TJMBMDMGMK	067/548	MRSA-107
6	10	t008	YHGFMBQBLO	008	MRSA-17
6	10	t008	YHGFMBQBLO	008	MRSA-29
6	10	t008	YHGFMBQBLO	008	MRSA-46
7	11	t051	YHFGFMBQBLO	008	MRSA-9
6	12	t1512	YPO	without alignment	MRSA-54
7	13	t051	YHFGFMBQBLO	008	MRSA-6
7	13	t051	YHFGFMBQBLO	008	MRSA-1
7	13	t051	YHFGFMBQBLO	008	MRSA-19
6	14	t008	YHGFMBQBLO	008	MRSA-67
6	15	t008	YHGFMBQBLO	008	MRSA-18
IV	15	t024	YGFMBQBLO	008	MRSA-114
6	15	t008	YHGFMBQBLO	008	MRSA-37
7	15	t024	YGFMBQBLO	008	MRSA-10
6	15	t024	YGFMBQBLO	008	MRSA-61
6	16	t008	YHGFMBQBLO	008	MRSA-32
6	16	t024	YGFMBQBLO	008	MRSA-75
6	17	t008	YHGFMBQBLO	008	MRSA-93
6	17	t008	YHGFMBQBLO	008	MRSA-121
6	17	t008	YHGFMBQBLO	008	MRSA-102
6	17	t008	YHGFMBQBLO	008	MRSA-92
6	18	t008	YHGFMBQBLO	008	MRSA-4
6	19	t064	YHGCMBQBLO	008	MRSA-41
7	20	t121	YHFMBQBLO	008	MRSA-5
6	21	t037	WGKAOMQ	012	MRSA-116
6	22	t008	YHGFMBQBLO	008	MRSA-34
6	22	t008	YHGFMBQBLO	008	MRSA-100
7	23	t008	YHGFMBQBLO	008	MRSA-21
5	24	t041	TO2MBMDMBMDMGMK	067/548	MRSA-27
5	25	t041	TO2MBMDMBMDMGMK	067/548	MRSA-56
5	26	t041	TO2MBMDMBMDMGMK	067/548	MRSA-76
5	27	t001	TO2MBMDMGMK	067/548	MRSA-33
5	28	t041	TO2MBMDMBMDMGMK	067/548	MRSA-39
5	28	t041	TO2MBMDMBMDMGMK	067/548	MRSA-85
5	29	t001	TO2MBMDMGMK	067/548	MRSA-118
5	30	t001	TO2MBMDMGMK	067/548	MRSA-42
VII	31	t001	TO2MBMDMGMK	067/548	MRSA-12
5	31	t001	TO2MBMDMGMK	067/548	MRSA-2
5	32	t041	TO2MBMDMBMDMGMK	067/548	MRSA-111
5	33	t1525	TO2MBMDMB	without alignment	MRSA-89
6	34	t030	WGKAQQ	012	MRSA-51
6	34	t030	WGKAQQ	012	MRSA-101
6	34	t030	WGKAQQ	012	MRSA-26
6	35	t030	WGKAQQ	012	MRSA-30
6	36	t074	WFGKAOMQ	012	MRSA-79
5	37	t1508	WKBPE	without alignment	MRSA-64
4	38	t1524	WGKOAKAKAOKAOMQQ	without alignment	MRSA-106
5	39	t002	TJMBMDMGMK	067/548	MRSA-43
2	40	t1522	XKAKBEME	without alignment	MRSA-91
2	40	t026	XKB	excluded	MRSA-95
I	40	t1709	YHGFMBQBLQ	without alignment	MRSA-16
2	40	t1510	XKAKBEMBKE	without alignment	MRSA-45
VI	40	t370	A2BMBKB	singleton	MRSA-81
2	40	t230	XKAKB	no founder	MRSA-69
2	41	t038	XE3BBEMBKB	singleton	MRSA-55
2	41	t026	XKB	excluded	MRSA-71
1	41	NT		without alignment	MRSA-7
2	41	t015	XKAKBEMBKB	singleton	MRSA-65
2	41	t004	A2AKEEMBKB	no founder	MRSA-25
2	42	t026	XKB	excluded	MRSA-77
1	43	t040	A2AKEMBKB	no founder	MRSA-11
8	43	t045	TMDMGMK	no founder	MRSA-49
2	43	t004	A2AKEEMBKB	no founder	MRSA-86
2	43	t040	A2AKEMBKB	no founder	MRSA-108
2	44	t1523	XKAKEEMBB	without alignment	MRSA-109
10	45	t595	UJ2GMKKPNSNSG	no founder	MRSA-50
10	45	t355	UJ2GMKKPNSG	no founder	MRSA-112
9	46	t044	UJGBBPB	singleton	MRSA-68
5	46	t044	UJGBBPB	singleton	MRSA-70
VIII	46	t044	UJGBBPB	singleton	MRSA-14
7	47	t037	WGKAOMQ	012	MRSA-20
3	48	t018	WGKAKAOMQQQ	012	MRSA-48
4	48	t391	WQ	excluded	MRSA-74
3	49	t007	WGKKKKAOM	singleton	MRSA-96
3	50	t012	WGKAKAOMQQ	012	MRSA-113
1	51	t1081	XKAX2BMB	no founder	MRSA-8
4	52	t019	XKAKAOMQ	singleton	MRSA-60
5	53	t016	TJEJNCMOKKR	singleton	MRSA-82
5	53	NT		without alignment	MRSA-87
5	53	t032	TJJEJNF2MNF2MOMOKR	singleton	MRSA-28
5	53	t032	TJJEJNF2MNF2MOMOKR	singleton	MRSA-57
5	53	t1251	TJJEKKKR	without alignment	MRSA-115
6	54	t400	YHGFMBBLO	008	MRSA-35
5	54	t032	TJJEJNF2MNF2MOMOKR	singleton	MRSA-53
5	55	t1526	TDNCMOKR	without alignment	MRSA-104
II	56	t163	ZDMDMA3KB	singleton	MRSA-94
3	56	t007	WGKKKKAOM	singleton	MRSA-110

FIG. 1. Dice cluster analysis of PFGE-generated fingerprints of the 106 MRSA strains. Corresponding data from PFGE, DiversiLab, *spa* typing including Kreiswirth nomenclature, and *spa* clonal clustering are included.



potential adoption of this method in a routine setting at our institution. Furthermore, we analyzed six USA300 strains collected in our hospital by applying these methods.

(These results were presented in part at the European Congress of Clinical Microbiology and Infectious Diseases, 10 to 13 April 2010, Vienna, Austria.)

MATERIALS AND METHODS

Bacterial isolates. The University Hospital Basel is a tertiary 950-bed institution serving the northwestern part of Switzerland with a population of approximately half a million people. Surveillance of MRSA began in 1992 (20), and each nonrepetitive clinical MRSA isolate has been typed by PFGE and stored since 1994. From this collection, we investigated a total of 106 MRSA isolates differing by at least one band within the PFGE pattern (8). The strains were obtained between 1994 and 2006. We also analyzed six USA300 strains from our hospital collected between 2003 and 2006 (33). Four of these USA300 isolates were part of the 106-strain set.

PFGE. The procedure was performed as previously described by Strandén et al. (27) using the restriction endonuclease SmaI. The percent similarities were identified derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. A similarity cutoff 80% was selected to define the PFGE type clusters as reported previously by McDougal et al. (18).

***spa* typing and *spa* clonal cluster analysis.** Both methods were carried out as described previously (13, 19). Nucleotide sequences were analyzed by using Ridom StaphType software and synchronized with SpaServer (www.spaserver.ridom.de). Clustering analysis into *spa* clonal complexes (*spa*-CC) was carried out by using the algorithm BURP with default parameters (28).

Typing with the DL system (rep-PCR). The MRSA strains were typed using the DiversiLab kit (DL; bioMérieux, Geneva, Switzerland) as recommended by the manufacturer. First, DNA extraction was performed by using an UltraClean microbial DNA isolation kit, a manual procedure using column purification. The DNA amount was subsequently measured by using a Nanodrop 2000 (Witec, Lucerne, Switzerland), and rep-PCR was executed with a *Staphylococcus* kit from DL, adding 25 to 50 ng of genomic DNA/μl.

The PCR amplification was performed on a Veriti thermal cycler (Applied Biosystems, Rotkreuz, Switzerland) with an initial denaturation at 94°C for 2 min; followed by 35 cycles of 30 s at 94°C, 30 s at 45°C, and 90 s at 70°C; and a final extension at 70°C for 3 min.

For analysis of the PCR products, we used an Agilent 2100 Bioanalyzer (Agilent Technologies) provided from bioMérieux in which amplified fragments of various sizes (from 150 to ~5,000 bp) were separated electrophoretically and detected by using a microfluidic labchip. On one chip, a total of 12 samples can be loaded in addition to a molecular size marker. Loading volume per sample was 1 μl. The electrophoresis data were analyzed by the internet-based DiversiLab software (version 3.4). It calculated a dendrogram based on the Pearson correlation coefficients and creates a scatter plot and a virtual gel image. The relatedness was determined by cluster analysis according to guidelines provided by the manufacturer. The strain clustering was defined as a minimum of 95% similarity, with a difference of up to one band in the dendrogram as described previously (31).

Discriminatory power and concordance of typing methods. To assess quantitative data of discriminatory power and concordance of the typing methods used in our study, we applied the software EpiCompare from Ridom (http://www.ridom.de/epicompare). The discriminatory power of each typing method was assessed using Simpson's index of diversity (SID) calculating the probability that two unrelated strains sampled from the test population will be placed into different typing groups. An index of >0.90 is desirable if the typing results are to be interpreted with confidence (15). The 95% confidence intervals of the SID values were calculated as described previously (11).

The quantitative concordance between typing methods was analyzed by using adjusted Rand and Wallace coefficients (3). The Wallace index indicates the probability that two strains classified as the same type by one method are also classified as the same by another method. A high Wallace coefficient shows that

FIG. 2. Cluster analysis and virtual gel image from DiversiLab-generated fingerprints of the 106 MRSA strains, including corresponding typing data from *spa* typing (class 1) and *spa* clonal clustering (class 2). Colored marks indicate the *spa* clonal clustering results.

TABLE 1. Discriminatory power of each typing method

Method	No. of strains	No. of types	No. of strains of major type (%)	SID (95% CI)[a]
PFGE	106	56	6 (5.7)	0.982 (0.976–0.988)
DL[b]	106	18	29 (27.4)	0.846 (0.807–0.885)
spa typing	104	47	15 (14.4)	0.950 (0.935–0.975)
spa-CC	77	18	27 (35.1)	0.757 (0.694–0.820)

[a] SID, Simpson's index of diversity; CI, confidence interval.
[b] DL, DiversiLab method.

the result obtained by a given typing method could have been predicted by the other method (3).

RESULTS

PFGE. The 106 strains grouped into a total of 56 PFGE clusters (1 to 56) using a cutoff 80% based on Dice coefficients from the PFGE data. The highest similarity rate was 96.6% between MRSA strains 93 and 121. The PFGE type with the highest number of isolates was PFGE type 40 with 6 strains (5.7%) (Fig. 1).

***spa* typing and *spa*-CC analysis.** *spa* typing differentiated the collection into 47 *spa* types. Two strains were not typeable, resulting in a typeability of 98.1%. The most common *spa* types were t008 ($n = 15$), t002 ($n = 13$), and t041 ($n = 5$).

The typeability of the *spa* clonal complex (*spa*-CC) analysis was 72.6% ($n = 77$). Three *spa*-CC were obtained: *spa*-CC 067/548 ($n = 27$), comprising *spa* types t001, t002, t041, t067, and t548; *spa*-CC 008 ($n = 26$), including *spa* types t008, t024, t051, t064, t121, and t400; and *spa*-CC 012 ($n = 9$), including *spa* types t012, t018, t030, t037, and t074. Fifteen *spa* types were singleton, twelve were without alignment, eleven had no founders, and four were excluded (Fig. 1).

DL typing (rep-PCR). Based on a minimum of 95% similarity with a difference of up to one band in the dendrogram, DL typing separated the 106 MRSA isolates into 10 distinct clusters (1 to 10) and 8 singleton patterns (I to VIII), resulting in a typeability of 100%. The DL clusters represented the number of strains as follows: DL 1 ($n = 3$), DL 2 ($n = 12$), DL 3 ($n = 4$), DL 4 ($n = 3$), DL 5 ($n = 29$), DL 6 ($n = 25$), DL 7 ($n = 8$), DL 8 ($n = 10$), DL 9 ($n = 2$), and DL 10 ($n = 2$). The five largest DL clusters represented 84 (79%) strains (Fig. 2).

Concordance between DL typing and *spa* clonal clustering. *spa*-CC 008 and *spa*-CC 012 ($n = 23$, 85.2%) shared mainly 2 DL clusters (DL 6 and 7), while *spa*-CC 067/548 strains ($n = 32$, 91.4%) mainly clustered into two distinct DL patterns (DL 5 and 8) (Fig. 1 and 2).

Discriminatory power and quantitative concordance. We determined the SID values of the four typing methods. PFGE had the highest discriminatory power, followed by *spa* typing, DL typing, and *spa*-CC (Table 1).

Quantitative analysis of concordance showed, as calculated by using the adjusted Rand index, the highest value between *spa* typing and *spa* clonal clustering, followed by DL typing and *spa* clonal clustering. Low quantitative concordance was found between DL typing and PFGE, as well as between *spa* clonal clustering and PFGE (Table 2).

As calculated by using Wallace coefficients (W), *spa* type had high probability to predict *spa*-CC (W = 0.970), followed by PFGE type to predict *spa*-CC (W = 0.836) and *spa* type to predict DL type (W = 0.640). Low Wallace coefficients were found for DL type and *spa*-CC to predict PFGE type (Table 2).

USA300 strains. PFGE type 17 represented the four USA300 strains within the PFGE clustering of the 106 isolates sharing a similarity of 87.2% (Fig. 1).

A similarity of 87.7% was obtained from our six USA300 strains in separate PFGE analysis. The six isolates grouped homogeneously into *spa* type t008 and *spa*-CC 008. DL typing clustered the six isolates into one DL type (DL 6). MRSA-123 represented the previously published (33) USA300 strain (Fig. 3).

DISCUSSION

An ideal typing system to characterize MRSA should be easy and fast to perform, as well as possessing sufficient discriminatory power, high reproducibility, and low cost. Furthermore, the results should be comparable in different hospitals and reference laboratories.

In the present study, we compared the performance of a new kit-based rep-PCR typing method to the reference methods SmaI PFGE and *spa* typing, including *spa* clonal cluster analysis. A collection of nonrepetitive, well-defined MRSA strains ($n = 106$) from our hospital was investigated.

We addressed the concordances of typing methods and found moderate concordance between *spa* clonal clustering and DL typing. *spa*-CC 008 and *spa*-CC 012 ($n = 23$, 85.2%) shared mainly 2 DL clusters (DL 6 and 7), while *spa*-CC 067/548 strains ($n = 32$, 91.4%) mainly clustered into 2 distinct DL patterns (DL 5 and 8) (Fig. 1 and 2). This observation was confirmed by the quantitative determination of concordance, calculating the adjusted Rand indexes. Beside the high value of concordance for *spa* typing and *spa* clonal clustering, which is method dependent, an adjusted Rand coefficient of 0.387 was found for DL typing and *spa* clonal clustering, followed by very similar coefficients for DL typing and *spa* typing, as well as PFGE and *spa* typing. In agreement with the findings of Te

TABLE 2. Concordances of typing methods using adjusted Rand and Wallace coefficients[a]

Method	Adjusted Rand index				Wallace coefficient			
	PFGE	*spa* typing	*spa*-CC	DL	PFGE	*spa* typing	*spa*-CC	DL
PFGE						0.457	0.836	0.540
spa typing	0.232				0.174		0.970	0.640
spa-CC	0.088	0.410			0.065	0.320		0.465
DL	0.083	0.236	0.387		0.063	0.187	0.587	

[a] DL, DiversiLab method.

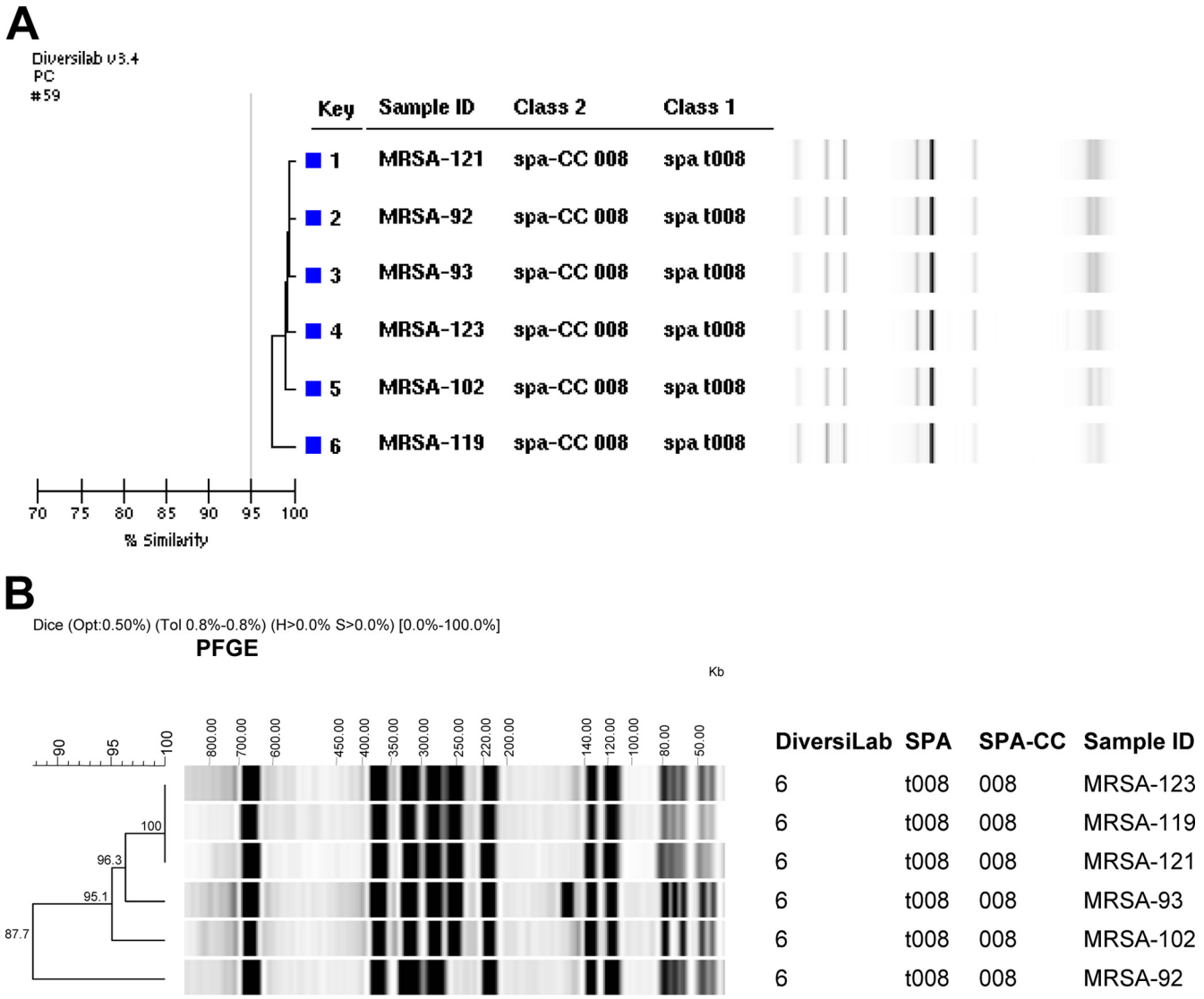


FIG. 3. Typing of the six USA300 isolates. (A) DiversiLab dendrogram, virtual gel image, and corresponding *spa* type and *spa* clonal cluster. (B) PFGE dendrogram and fingerprints using Dice cluster analysis. Also shown are corresponding DiversiLab, *spa* type, and *spa* clonal cluster data. MRSA-123 represents the USA300 strain published previously (33).

Witt et al. (32), we found a low level of concordance between DL typing and PFGE.

The *spa* types assigned to *spa*-CC 012 belong to different clonal complexes as defined using MLST. *spa* types t012 and t018 belong to CC30, whereas *spa* types t030 and t037 are part of CC8/239. As can be seen in Fig. 1, our *spa* t030 and t037 strains homogeneously cluster within the *spa*-CC 008 strains according to rep-PCR typing. This observation can be explained by the hybrid origin of ST239-MRSA (23).

The results of discrimination calculated by using SID values confirmed PFGE as a highly discriminative method. The very high values of 0.980 for PFGE and 0.950 for *spa* typing can be explained by the composition of our strain collection, which only included MRSA isolates differing by at least one band within the PFGE pattern. Fenner et al. (8) analyzed in an epidemiological study a high portion of our collection with *spa* typing and *spa* clonal clustering. They found no predominant *spa* type, suggesting a great genetic diversity among our strains.

Similarly Cookson et al. (5) reported high SID values of 0.919 for PFGE and 0.913 for *spa* typing analyzing 98 strains of the HARMONY collection from 11 European countries, and Te Witt et al. (32) investigated a total of 93 isolates of the identical HARMONY library and calculated SID coefficients of 0.905 for PFGE and 0.860 for DL typing. Comparably low SID values for in-house rep-PCR were also reported by Deplano et al. (6). Importantly, an SID of DL typing of greater than 0.90 is desirable (33). However, we calculated an SID value of 0.846 using this technique, which argues against the use of DL typing as a standalone typing technique. This view is supported by two other reports (24, 31), which compared DL typing to PFGE analyzing well-defined MRSA strains and found DL typing to be less discriminative. In both publications

the authors recommend that DL typing be used to screen isolates, followed by testing strains that share the same rep-PCR type with a more discriminative method.

Further evaluation of the DL typing method compared to PFGE was performed by Shutt et al. (26). They investigated 19 MRSA outbreaks and summarized that the performance of DL typing was comparable to PFGE. In 2009, Tenover et al. (31) tested 105 MRSA isolates of PFGE types USA100 to USA1000 with the DL typing system. DL typing grouped most of the USA100, USA200, and USA1000 types into unique clusters. However, most of the USA300 and USA500 types clustered together and could only be differentiated by using the pattern overlay function of the DL software. Recently, DL typing was used to characterize 14 strains of MRSA ST 398. This latter strain is not typeable by SmaI-PFGE. Evidently, DL typing was a reliable technique in determining genetic relatedness of ST 398 (10). Lastly, Church et al. (4) compared 10 prototype MRSA strains of known Canadian epidemic strains and 45 randomly selected MRSA strains with DL, *spa* typing, and PFGE. These authors found a high level of discrimination for *spa* typing across all PFGE types tested and concluded that DL typing is a good method for rapid outbreak screening but that MRSA strains which share the same DL or PFGE pattern should be distinguished by *spa* typing.

A limitation of our study is that we did not assess reproducibility of the DL typing method. Intra- and intertest reproducibility is an important feature of a typing method (29). However, the test is already standardized in a kit format, and data from three previous studies documented an excellent reproducibility of DL typing (24, 26, 32). This argues for reliable and reproducible data performed during the present study.

We confirm previous studies to demonstrate that DL typing is very rapid and relatively easy to perform (24, 26, 31, 32). Compared to PFGE, which is technically demanding, possesses a low throughput, and requires 3 to 5 days, DL typing has a high throughput and provides results within 1 day. Based on the kit format and web-based software, the DL method provides standardized results and generates user-friendly reports. The DNA extraction step of DL method was, however, rather laborious and might be replaced by a shorter, preferentially automated technique. We also observed the occasional formation of air bubbles as one microliter of DNA was loaded into the slots of the labchip (26, 32). This necessitates repeated testing, which increases both turnaround time and costs. A further limitation of the DL typing system is that the manufacturer does not provide definitive interpretation criteria for the test (24).

The *spa* typing analysis and DL typing have been comparable regarding the relative costs, speed, and expertise. In a very recent study (4), the calculated annual costs of DL and *spa* typing were similar. However, the technical requirements for *spa* typing are actually simpler than for DL typing, whereas data interpretation for *spa* typing is facilitated by interlaboratory comparison and universal nomenclature.

We also typed six isolates of MRSA USA300 which had previously been identified by PFGE. The USA300 clone is a community-acquired MRSA, an MRSA identified in previously healthy individuals with no recognized MRSA risk factors. In 2006, USA300 accounted for 97% of all MRSA isolates identified in adult patients with acute soft tissue and skin infections presenting to university-affiliated emergency departments in the United States (21). Reports of this clone in Europe are increasing (22) and have also been published previously by our institution (33). The six strains belonging to *spa* type t008 were all Panton-Valentine leukocidin (PVL) gene positive and easily met the criteria of USA300 clustering with a similarity cutoff >80%, as well as sharing the characteristic PFGE patterns (18). In DL typing, they homogeneously clustered into DL type 6. In contrast to Larsen et al. (17), we did not detect isolates belonging to a second clone of MRSA with the USA300 PFGE profile in our hospital. This clone occurring in Denmark belongs to *spa* type t024, does not possess PVL genes, and is mainly hospital acquired.

To conclude, the DL method proved to be rapid and easy-to-perform in contrast to PFGE. DL typing qualifies for initial screening during outbreak investigation. However, DL typing has limited discriminatory power and therefore should be complemented by more discriminative techniques such as *spa* typing or PFGE in isolates which share identical DL patterns.

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